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TITLE

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PAGE 2

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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: WILLIAM R. ALONSO

Serial No.: Unassigned

Filed: Unassigned

Title: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

TRANSMITTAL LETTER

EXAMINER: Unassigned

ART UNIT: Unassigned

Box: PATENT APPLICATION
Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Transmitted herewith for filing are the following:

1. Application for United States Patent, including 23 pages of Specification and Claims, an Abstract and 1 sheets of drawings of Applicant: WILLIAM R. ALONSO for: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN.

2. An Assignment document is enclosed, together with a Request for Recordation.

3. The filing fees are calculated as follows:

For	Number Filed	Number Extra	Rate	Fee
Basic Fee				$730.00
Total Claims	24	4	22	88.00
Independent Claims	3	0		0
Multiple Dependent Claims	0	0		0
			TOTAL	$818.00

4. The Commissioner is hereby authorized to charge the above $818.00 filing fee and any other filing fees which may be required or credit any overpayment to Deposit Account No. 03-4000. A duplicate of this sheet is enclosed.

5. An executed Declaration for Patent Application is enclosed.

6. Address all future communications to:

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Patent
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: WILLIAM R. ALONSO

Serial No.: UNASSIGNED

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Title: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

Examiner: UNASSIGNED

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CERTIFICATE OF EXPRESS MAIL

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I hereby certify that the enclosed Patent Application, Figures, Declaration and Power of Attorney, Assignment, Recordation Form Cover Sheet and Assignment Transmittal Letter are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Luajuana Riley
Luajuana Riley

Luajuana Riley
Signature

September 22, 1995
Date

32211

Patent MSB-7232

APPLICATION FOR UNITED STATES PATENT

Title: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

Inventor: William R. Alonso

BACKGROUND OF THE INVENTION

Field This invention generally deals with an intravenously injectable immunoglobulin product, and more specifically deals with an intravenously injectable immune serum globulin (IGIV) which has been subjected to a virus inactivation step and which has a low level of anticomplement activity.

Background Early pharmaceutical preparations of immune serum globulins could not be administered intravenously due to an unacceptably high incidence of adverse reactions. These adverse reactions were associated with a decrease in serum complement levels, apparently caused by complement binding to the administered gamma globulin. (1) The ability of gamma globulin to bind complement, or its anticomplement activity (ACA), is greatly increased as a result of denaturation brought about during the fractionation procedure. Several approaches have been taken to address the problem of rendering ISG safe for intravenous administration. (See (2) and references therein). Tenold reported a method of preparing an immune serum globulin (ISG) with low ACA which could be administered by intravenous injection. (2, incorporated herein by reference). The Tenold '608 process requires formulating the ISG at low ionic strength (preferably less than about 0.001) and at low pH (3.5-5.0).

Other methods of preparing intravenously injectable immune serum globulin (IGIV) have been reported, including stabilizing with carbohydrates such as maltose (3). A process including incubation of ISG at pH 4.0 at 37° C (4) results in a product with low ACA which may be administered by intravenous injection; however, upon storage the product regains its high ACA. IGIV has also been prepared by covalent modification of the ISG, for example by proteolysis (5) or by reduction of disulfide linkages followed by reaction with a blocking agent (1,6).

Antibody preparations, since they are isolated blood products, have an inherent hazard of transmitting virally-mediated diseases. Inactivation of viruses is an important step in producing safe and effective blood products. U.S. Patent 4,540,573 to Neurath et al., which is incorporated herein by reference, describes a viral inactivation process using a trialkyl phosphate and detergent process (hereinafter, the solvent/detergent process, or SD process). (7) That solvent/detergent method has gained acceptance as being efficacious in the inactivation of lipid-enveloped viruses with limited adverse effects on biological activity or blood product profile. (8, 15; See also 12 for a discussion of various viral inactivation processes).

Current antibody preparations on the market generally have been regarded as safe with respect to viral contamination. (9) This is thought to be due to features of the fractionation processes used to isolate these blood products. However, it would be desirable to further ensure the safety of the antibody preparations by including a distinct viral inactivation step in the production process. Successful reduction of viral activity in an IGIV solution was reported using several different methods of viral inactivation for a variety of viruses. (16, 17) A process for preparation of immunoglobulins substantially free of retrovirus has been reported involving incubation of ISG under controlled conditions of time, temperature, and pH. The process entails isolating ISG via a cold ethanol plasma fractionation process and then storage of the ISG at one of two storage conditions: (a) at pH≤ 4.25 at a temperature of 27° C for at least three days, or (b) at pH ≤ 6.8 at a temperature of 45° C for at least six hours. (10).

We have found that using the SD process to treat ISG preparations, especially those subsequently formulated according to the Tenold '608 patent, results in a product with an acceptable viral inactivation but with unacceptably high levels of ACA. Elevated ACA levels were always detected at the sterile bulk stage (i.e., after compounding as 5% or 10% IGIV and filtration with 0.2 μm sterile filters) of all tri-n-butyl phosphate (TNBP)/detergent treated

IGIV preparations regardless of process scale. Preparations of ISG with high ACA levels are not suitable for intravenous injection and instead must be administered via other routes, e.g. intramuscular (IM) injection. However, IGIV preparations are more desirable since they are immediately available in the bloodstream and are not subject to loss associated with IM injection. It is thus desirable to have an IGIV product which is both low in ACA and has been subjected to a viral inactivation step.

SUMMARY OF THE INVENTION

The invention is a method for producing an intravenously injectable immune serum globulin (IGIV) preparation with low anticomplement activity which has been chemically treated to render it substantially free of lipid-enveloped viruses. The method comprises a solvent/detergent viral inactivation step followed by an incubation step. We have discovered that the incubation step is necessary to achieve an acceptable level of ACA low enough to allow the ISG to be administered by intravenous injection. The incubation step should be conducted under controlled time, pH, temperature, and ionic strength. Preferably, the pH should be maintained between about 3.5 and about 5.0, the temperature should be within a range of about 2 to about 50° C, and the ionic strength should be less than about 0.001 M. In a preferred embodiment the ACA of the ISG preparation decreases gradually over a period of at least about ten days when the ISG is maintained at a pH of about 4.25 at low ionic strength (less than about 0.001 M) and the viral inactivation step (in a model system) results in a substantial reduction (i.e. at least 4 logs) in the titer of lipid enveloped viruses.

BRIEF DESCRIPTION OF THE FIGURE

FIGURE 1

The Figure shows a comparison of the typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation of the present invention.

SPECIFIC EMBODIMENTS

Materials and Methods

The starting material for the process of this invention is unmodified human immune serum globulin. In the specification and claims the term "immune serum globulin" is used to define the substance also referred to in the literature variously as gamma globulin, IgG and immunoglobulin G. It consists predominantly and preferably of at least about 85 percent of the 7S species of gamma globulin, which has a molecular weight of about 160,000. Any remainder is preferably 9S species, with a molecular weight of about 300,000. Both standard immune and hyperimmune serum globulins, e.g., tetanus, rabies and hepatitis immune serum globulins, can be employed, the solvent/detergent treated product being immune and hyperimmune ISG, respectively. Thus, a suitable starting material for the process of this invention is Cohn's Fraction II or Fraction III filtrate. (See Refs. 13, 14.)

Fraction II, by ultracentrifugation studies, is predominantly (about 85 percent) the 7S (sedimentation constant of 7) species of gamma globulin with an average molecular weight of 160,000. The remaining protein is essentially 9S material with a M.W. of about 300,000. Wet Fraction II paste (approximately 30 percent solids) is commonly lyophilized to obtain dry ISG powder which is then dissolved and prepared for intramuscular injection as a 16.5 percent sterile solution. Either the wet Fraction II paste or the dry ISG powder is a suitable starting material for the process of this invention.

Gamma globulin obtained by any process which has essentially the same composition of protein components as found in the Cohn Fraction II or Fraction III filtrate can be used as starting material in the present process. Both standard immune serum globulin and hyperimmune serum globulin can be employed as starting materials. As is well known, the

latter is produced from plasma or serum obtained from selected donors who have much higher titers for a specific antibody than is normally found in the average population. These donors have either been recently immunized with a particular vaccine or else they have recently recovered from an infection or disease. These high titer sera or plasmas are pooled and subjected to the usual Cohn fractionation procedures up to the point of isolating Fraction II.

Furthermore, because the amount of antibody required to achieve a desired immunological response is substantially less when administered intravenously, it will be apparent the intravenous dose will be substantially less than the intramuscular dose which will produce the same serum antibody titer. Thus, the dose of intramuscular ISG and hyperimmune serum globulin must be higher than that required to achieve the same serum antibody titer when globulin of the same antibody activity is administered intravenously.

The starting wet paste or lyophilized ~~power~~ powder is dissolved in a volume of water or other physiologically-acceptable carrier to provide a protein solution of a concentration of about 0.5-20% preferably about 5 to 10 percent. If Fraction III filtrate is employed, the aqueous solution must be concentrated by conventional techniques to the desired protein concentration. Any protein concentration may be used in this method; however, the above range is preferred from a practical standpoint.

After the protein has been dissolved or concentrated, the solution is adjusted to a pH of about 3.5 to 5.0 preferably about 3.8 to 4.2, by addition of a physiologically-acceptable acid such as hydrochloric acid. In general, the pH is adjusted to a point whereat the monomeric material in the protein solution is maintained at a maximum. However, the pH must not be so low as to result in gelation. The temperature should not be harmful to the ISG material. Good results are obtained within the temperature range of about 0 - 20° C. It is not necessary to hold the so-adjusted material for any period of time prior to the next step; however, the material may be held, if desired, without detrimental effects.

5

The protein solution at the appropriate pH (preferably 3.8 - 4.2) may be diafiltered with at least 4 volume exchanges of water to reduce the alcohol concentration from approximately 17% (Filtrate III) to about 2% alcohol. The efficacy of solvent/detergent as a viral inactivation method is much better at or above ambient temperatures; however, high concentrations of alcohol at these temperatures will denature the IgG molecules. Thus, this inactivation must be performed in low alcohol concentration.

Next, the protein concentration of the so-treated material is adjusted to the level desired for incubation with TNBP/detergent, generally less than 10% protein for maximum viral inactivation. This adjustment is accomplished by conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Prior to addition of TNBP/detergent, the pH may be adjusted within a wide range, depending on the detergent to be used. With Tween 80, the pH may be as low as 3.5, where the IgG starts becoming unstable. With cholate, the pH is adjusted to within the range of 5.0 - 6.4, preferably about 5.6, prior to addition of TNBP/detergent. Satisfactory cholate solubility during incubation was achieved by adjusting the immunoglobulin solutions to a pH of 5.5 or higher prior to addition of TNBP and sodium cholate. Adjusting the IgG solution to pH values lower than 5.5 is not suitable because the solubility of sodium cholate is highly dependent on pH (cholic acid pK = 6.4), with poor solubility at pH 5.5 or lower. Furthermore, maximum viral inactivation during incubation with TNBP/cholate was observed at pH values less than 6.0 in experiments which employed model viruses spiked into IgG solutions. The inactivation of HIV-1 and BVDV (bovine viral diarrhea virus, which is employed as a model for hepatitis C) was accelerated at pH 5.8, with inactivation to the detection limit occurring in 1-2 hours, whereas inactivation to the detection limit required a minimum of 6 hours when pH 7 conditions were used.

Next, the TNBP/detergent is added to the protein solution (preferably less than 8% [w/w], pH 5.8) mixed thoroughly, and then incubated above ambient temperatures, for example 30° C, with continuous agitation or mixing. Target TNBP/cholate levels for optimal viral inactivation during the incubation step should be > 3 mg/mL TNBP and > 2 mg/mL cholate as defined by Edwards et al. (8) Moreover, for effective viral inactivation, it is important that the solution is essentially free of particulates in order to facilitate thorough mixing of solvent/detergent and IgG solution. After incubation with TNBP/cholate under these conditions, greater than 5.2 $\log_{10}$ reduction of HIV-1 and greater than 4.0 $\log_{10}$ reduction of BVDV were detected.

After completing the incubation which provides the viral inactivation, the solvent and detergent molecules must be removed in order to achieve a final product with low levels of residual TNBP and cholate which would be suitable for intravenous administration. Generally, procedures to remove detergent are also effective in removing TNBP, and vice versa. Very low levels of TNBP and cholate in the final container can be achieved by a combination of filtration, diafiltration and hydrophobic chromatography. After completing the incubation, the majority of cholate (and TNBP) can be removed from the protein solution by filtration, providing the solution had been previously adjusted to a lower pH value such as 4.0, because sodium cholate is sparingly soluble in aqueous solutions at such pH values. Moreover, all processing steps which follow the solvent/detergent incubation are performed at lower pH values (i.e., 4.0) because IgG molecules are more stable at pH values between 3.5 - 5.0, in low ionic strength solutions. (2) Thus, after incubation with TNBP/cholate, the protein solution is adjusted to approximately pH 4.0 and incubated at 0 - 8° C in order to promote cholate precipitation. Next, filtration is employed to remove the precipitated cholate from the IgG solution.

The so-treated solution is diafiltered with at least four volume exchanges of water to reduce the ionic strength and to remove additional TNBP and cholate. After or during the above treatment, the pH is measured and maintained within the range of about 3.5 - 5.0. The protein concentration of the so-treated material is adjusted to 10 - 30%, usually 13% (w/v) by employing conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Again the pH of the preparation is maintained within the range of about 3.5 - 5.0, preferably about 3.8 - 4.2.

In the present invention, hydrophobic chromatography is employed to remove the TNBP and cholate not eliminated by the filtration and diafiltration steps, and thus provide a final product with low levels of residual TNBP and cholate which is suitable for intravenous administration. Hydrophobic chromatography is a method for TNBP removal from protein solutions that has fewer drawbacks and limitations than other available methods such as oil extraction, ion exchange or affinity chromatography. In part, this is because the protein of interest (IgG) remains in solution throughout the TNBP removal process. Polystyrene-based resins (typically PLRP-S from Polymer Laboratories, Amherst, MA) were used to remove the solvent/detergent from solution, as we have found the polystyrene-based resins to be superior to other resins, such as silica-based C-18 resins.

Next, the ISG preparation is adjusted to 5% or 10% protein, and treated to render it tonic, i.e., to render it compatible with physiological conditions, or render it physiologically acceptable upon injection. In a preferred embodiment, the tonicity is adjusted to about 230 to about 490 mosmol/kg solvent. More preferably, the tonicity range is from about 250 to about 350 mosmol/kg solvent, and most preferably the tonicity range is from about 260 to about 325 mosmol/kg solvent. The 5% formulation (5% IGIV) is made tonic by the addition of 10% maltose. The 10% formulation contains 0.2 M glycine in order to achieve an isotonic preparation without large quantities of sugar. The product with either formulation

(Gamimune®N 5% or Gamimune®N 10%) experiences shifts in molecular distribution (antibody aggregation) when the ionic strength of the low pH solution is increased. Therefore, sodium chloride, which is often used to achieve tonicity, should not be used.

The so-treated solution is incubated at pH 4.25 under low ionic strength conditions (NLT 21 days at 20 - 27° C preferred) in order to provide a lowering of ACA levels. The ionic strength is determined according to Perrin (18), and in a preferred embodiment the ionic strength should be less than about 0.001. Elevated ACA levels were always detected at this stage of all TNBP/cholate treated IGIV preparations (regardless of process scale); however, ACA levels are gradually lowered by incubation at pH 4.25 under low ionic strength conditions (Tables 3, 5 - 7). While there is no strict rule for determining when the ACA level is low enough to be an acceptable level suitable for intravenous administration, IGIV preparations should have ACA levels as low as possible.

The Figure depicts the typical average reduction of ACA observed in 5% IGIV solutions following SD treatment. For a 5% ISG formulation the acceptable level suitable for intravenous administration preferably would be less than about 45 CH_{50} units/mL, and more preferably less than about 30 CH_{50} units/mL. For a 10% ISG formulation, the acceptable level suitable for intravenous administration preferably would be less than about 60 CH_{50} units/mL, and more preferably less than about 45 CH_{50} units/mL. As used herein, one unit of ACA activity (one CH_{50} unit) is defined as the amount of protein capable of activating 50% of the complement in an optimally titered complement and red blood cell/hemolysin system. The assay measures the amount of complement that is bound by the mixture of standardized amounts of complement and protein. See refs. 19-20 for a discussion of the assay. Briefly, red blood cells that have been sensitized by preincubation with red blood cell antibodies are added to the complement/protein mixture. In the presence of free complement (not already bound by the protein) these sensitized cells will lyse, releasing hemoglobin which can be quantitated as a measure of the degree of lysis. In parallel, sensitized red blood cells are also added to a buffer control-complement mixture, whose degree of lysis is defined as 100%. The

difference between the actual amount of complement needed to give 100% lysis and the amount of complement remaining unbound in the presence of protein equals the amount of complement actually bound by the protein, or anticomplement activity.

Results

Anticomplement activity of ISG resulting from viral inactivation process

To establish the effect of the SD viral inactivation process on solutions containing ISG which are formulated according to the Tenold '608 patent, the experiments depicted in Table 1 were performed. The starting material (SM) was Cohn process filtrate III which had been ultrafiltered to about 5% protein and then diafiltered with four volumes of water.

In the control experiment, incubation (-)/SD (-), the SM was not subjected to any incubation or solvent/detergent treatment. In the incubation (+)/SD (-) experiment, the pH of the SM was adjusted to 7.0, the solution was incubated at 30° C for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/ SD, TNBP & Tween 80 (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL Tween 80 were added to the solution, the solution was incubated at 30° C for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/SD, TNBP & cholate (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL cholate were added to the solution, the solution was incubated at 30° C for ten hours, and then the pH was reduced to 4.0. The solutions in each experiment were then diafiltered with four volumes CWFI (cold water for injection) and concentrated by ultrafiltration. After addition of dry maltose to 10% w/v, the 5% IGIV solution (pH 4.25) was filtered through a 0.2 μm filter.

Table 1
Anticomplement activity in 5% IGIV produced by variations of the Solvent/Detergent IGIV Process

	ACA (CH_{50}/mL)
Control (no solvent/detergent treatment, no 30° C incubation)	25
Incubate at 30° C for 10 hr (no solvent/detergent)	22
Incubate at 30° C for 10 hr NLT 3 mg/mL TNBP NLT 2 mg/mL Tween 80	68
Incubate at 30° C for 10 hr NLT 3 mg/mL TNBP NLT 2 mg/mL cholate	> 100

* These samples were assayed for ACA after final compounding according to the Tenold '608 patent, but they were not incubated at pH 4.25 and 22° C prior to analysis.

The results listed in Table 1 show that levels of ACA increased in IgG samples after incubation with TNBP/cholate or TNBP/Tween 80. ACA levels were not elevated in IgG samples that were incubated for 10 hr at 30° C in the absence of solvent/detergent. These results suggest that ACA levels of IGIV samples were not elevated by either processing manipulations or incubation for 10 hr at 30° C in the absence of solvent/detergent.

T/C130

Table 2
Anticomplement activity in 5% IGIV spiked with TNBP/Na cholate

	ACA (CH_{50}/mL)
5% IGIV, no TNBP/cholate	12
5% IGIV with 100 μg/mL TNBP, 100 μg/mL Na cholate	13

Furthermore, spiking experiments (with TNBP and Na cholate, Table 2) have demonstrated that the elevated anticomplement activity levels were not artifacts caused by carrying out the anticomplement assay in the presence of trace levels of TNBP/Na cholate. Thus, using the prior art SD process for viral inactivation of a solution containing ISG, subsequently formulated according to the Tenold '608 patent, yields a product which has high ACA and is unsuitable for intravenous administration. In a similar experiment, SD treated samples which were not incubated (Table 3, Initial Testing) had ACA levels greater than 100 units.

T/C131

Table 3
Reduction in Anticomplement activity of samples previously treated with TNBP/cholate

Sample	ACA (CH_{50}/mL)	
	Initial Testing (no incubation)	After incubation 6 wk. @ 5°C 3 wk. @ 22°C
RB21872-16	> 100	33
RB21872-17	> 100	34
RB21872-18	> 100	36
RB21872-20	> 100	27

B

However, when duplicate SD treated samples were incubated for extended periods of time (6 weeks at 5° C and 3 weeks at 22° C), the level of ACA was markedly reduced (Table 3, after incubation). This led to further investigation of this surprising observation.

Aggregate content of ISG exposed to TNBP/cholate

The samples of the previous experiment (Table 3, Initial Testing) were analyzed by size exclusion (gel permeation) HPLC immediately after compounding to determine the extent of aggregation of the IGIV at the initial time point. HPLC analysis shows nearly complete monomer content in the samples. (Table 4).

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Table 4
HPLC analysis of non-incubated 5% IGIV samples (Table 3 Initial)

Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
RB21872-16, initial	0.140	0.00	99.86	0.00
RB21872-17, initial	0.146	0.00	99.85	0.00
RB21872-18, initial	0.124	0.00	99.88	0.00
RB21872-20, initial	0.172	0.00	99.83	0.00

Previously, high IgG aggregate levels were shown to correlate with high anticomplement activity. However, results from analysis of the samples show the level of ACA in the samples to be greater than 100 units. (Table 3, 'Initial Testing') The HPLC analysis shows that the high ACA following the TNBP/cholate treatment was not due to the presence of aggregated IgG molecules.

Varied conditions of time and temperature

The SM was the same as in the previous experiment, and experimental conditions were similar with the following changes. The solutions were treated with TNBP/cholate at pH 7.0 and then were compounded to 5% IGIV, 10% maltose, pH 4.25, as above. The ACA was assayed immediately after final compounding, after a first incubation for nine days at 5° C, and after a second incubation for 21 days at either 22° C or 5° C. The results are presented in Table 5.

Table 5
ACA of TNBP/cholate treated IGIV samples

Sample Point	ACA (CH_{50}/mL)
Intermediate Samples	
Initial sterile bulk	> 100
Incubated 9 d. @ 5°C	> 100
Final Incubation	
21 d. @ 22°C	49
21 d. @ 5°C	71

In the initial sterile bulk sample, which was treated with TNBP/cholate at pH 7.0, the level of ACA was again greater than 100 units for the initial time point, confirming the observations noted in Table 3. Upon incubation at 5° C for nine days, the ACA remained greater than 100 units. The final incubation step at either 5° C or 22° C shows that the reduction in ACA is dependent on temperature, with faster reduction in ACA observed at higher temperatures.

Effect of pH during solvent/detergent treatment on ACA

ACA levels were evaluated after incubation with TNBP/cholate at pH 5.8 because better viricidal activity was observed at pH values less than 6.0. Generally, the non-incubated

sterile bulk samples of material incubated at pH 5.8 had lower ACA levels than the pH 7.0 samples, but the trend of lowering ACA upon incubation was repeated in the pH 5.8 samples. In fact, the ACA levels continue to decrease beyond the 21 day incubation in samples that initially had elevated ACA levels after incubation with TNBP/cholate at pH 5.8 (Table 6). As was previously noted for the samples incubated at pH 7.0, the lowering of ACA was not due to decreasing levels of aggregated IgG molecules because the material treated at pH 5.8 was essentially monomeric IgG prior to 22° C incubation (HPLC analysis, sample A4, Table 8).

Table 6
Sample A4 - ACA upon extended incubation

Incubation at 22°C (days)	CH_{50}/mL
0	122
10	73
19	55
25	56
28	45
30	40
34	39
41	33
48	30
55	29

Similar results were achieved with samples formulated to 10% IGIV, 0.2 M glycine in the sterile bulk stage. Upon incubation at low ionic strength at pH 4.25 for 10 and 21 days, the levels of ACA were seen to decline in both 5% IGIV samples and 10% IGIV samples. (Table 7) The decrease in ACA can thus be observed over a range of ISG concentrations and over a range of pH values for the solvent/detergent treatment. (Tables 3, 5, 7) HPLC analysis (Table 8) of the sterile bulk samples presented in Table 7 confirmed that the elevated ACA levels were not due to aggregation of ISG molecules.

T,C 17C

Table 7
ACA of samples treated with TNBP/cholate at pH 5.8

Sample	Sterile bulk (day zero) (CH_{50}/mL)	10 days incubation at 20 - 27°C (CH_{50}/mL)	21 days incubation at 20 - 27°C (CH_{50}/mL)
A1 (5% IGIV)	43	ND	10
A2 (5% IGIV)	31	14	15
A3 (5% IGIV)	44	15	12
A4 (5% IGIV)	122	73	55
B1 (10% IGIV)	>100	48	46
B2 (10% IGIV)	49	36	30
B3 (10% IGIV)	53	ND	37

Taken together, the above results suggest that ISG products which have been subjected to a solvent/detergent viral inactivation process resulting in an undesirable ACA increase can be made suitable for IV administration by incorporating an additional incubation step under the conditions described here to reduce the ACA to an acceptable level.

17

Table 8
HPLC Analysis of sterile bulk samples treated with TNBP/cholate at pH 5.8

Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
A2	0.140	0.00	99.86	0.00
A3	0.146	0.00	99.85	0.00
A4	0.124	0.00	99.88	0.00

CONCLUSION

The ACA increase resulting from the solvent/detergent treatment of the IGIV (antibody) solution appears to be an unavoidable secondary effect of TNBP/detergent treatment to inactivate viruses in the solution. I have discovered that by incubating the solution of IGIV at low pH (4.25) and low ionic strength (0.001 M) for a relatively long period of time (at least about 10 days), the ACA gradually decreases over the period of incubation.

The prior art discloses a method of producing IGIV (the Tenold '608 patent) using low pH and low ionic strength. The Tenold '608 method omits the viral inactivation step, and thus avoids the problem of increased ACA, but the possibility of viral activity remains. Unlike Tenold, incubation is an essential aspect of the present invention for reducing the ACA.

The Neurath et al. '573 patent teaches the solvent/detergent viral inactivation step. However, Neurath '573 does not mention controlling the pH and also does not mention any consequences of the process relating to ACA. Elevated ACA levels were detected at the sterile bulk stage of TNBP/cholate treated IGIV preparations. However, ACA levels decreased upon

incubation for at least about 10 days at pH 4.25, low ionic strength, and not less than about 20° C. (See Tables 5-7) The prior art describes several approaches to lowering ACA levels of purified IgG preparations, including removal of IgG aggregates. (11) IgG aggregates have been shown to activate the complement system in vivo. (1) In the present invention, however, lowering of IgG ACA was not due to decreasing levels of IgG aggregates because these TNBP/cholate treated IGIV preparations contained low levels of aggregated IgG (as measured by HPLC, Tables 4, 8) prior to incubation under such conditions.

It would be desirable to produce substantially virus-free IGIV, but following the prior art results in a product with an unacceptable level of ACA. Note that Tenold '608 states that the product is substantially free of ACA, but use of the SD process in conjunction with Tenold '608 does result in high levels of ACA: experimental results reported here show that treating ISG solutions with the SD process and then formulation according to the Tenold '608 patent leads to a product with high ACA. (See Tables 1, 3, 5-7) The surprising finding reported here is that a follow-up (terminal) incubation step lowers the ACA of the solvent/detergent treated solution. The typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation are compared in the Figure. The present invention thus includes a previously unobserved method of reducing the ACA by incubating under controlled conditions of pH, temperature, and ionic strength for a period of time, thus allowing the product to be administered by intravenous injection.

Mitra '714 does not suggest the use of a S/D process but, instead, reports that a relatively brief incubation of an ISG product under similar conditions results in a substantially virus free preparation. (10) However, employing incubation under such conditions to provide a lowering of anticomplement activity is a novel application of these incubation conditions which were previously employed in the IGIV process for inactivation of enveloped viruses.

The newly developed IGIV process reported here, which includes an additional internationally accepted viral inactivation procedure (treatment with TNBP/cholate), generates IgG preparations which have low ACA levels and are suitable for IV administration. The major advantage is that an IGIV product with improved safety can be obtained by a two-step process that includes a TNBP/cholate treatment for viral inactivation and incubation under conditions that afford low ACA levels that are suitable for IV administration.

The above disclosure is intended to illustrate the invention, and it is thought variations will occur to those skilled in the art. Accordingly, it is intended that the scope of the invention should be limited only by the claims below.

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7 Neurath, A. R. and Horowitz, B., U.S. Patent #4,540,573 (Sept. 10, 1985).

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19 Palmer, D. F. and Whaley, S. D., *Complement Fixation Test, in* Manual of Clinical Laboratory Immunology (Ed. N. R. Rose, et al., American Society for Microbiology, Washington, D.C., 1986) pp. 57-66.

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20

CLAIMS

What is claimed is:

1. A method of treating a solution of antibodies which may have virus activity, the method comprising

   a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in ~~a given~~ an increased level of anticomplement activity; and

   b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.

2. The method of claim 1, wherein the anticomplement activity is reduced to less than about 60 CH_{50} units/mL.

3. The method of claim 1, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

4. The method of claim 3, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 30 CH_{50} units/mL.

5. The method of claim 1, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 60 CH_{50} units/mL.

6. The method of claim 5, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

7. The method of claim 1, wherein the incubation is for at least about ten days.

8. The method of claim 1, wherein the pH is maintained within a range of about 3.5 to about 5.0.

9. The method of claim 1, wherein the temperature is maintained within a range of 2° C to 50° C.

10. The method of claim 1, wherein the ionic strength is less than about 0.001 M.

11. The method of claim 1, wherein at least about 99% of the antibodies are monomeric.

12. The method of claim 1, comprising the further step of adjusting the tonicity of the solution to a physiologic value under such conditions that the ionic strength is not appreciably altered.

13. The method of claim 12, wherein the tonicity of the solution is adjusted by adding a carbohydrate to the solution.

14. The method of claim 13, wherein the carbohydrate used is maltose.

15. The method of claim 12, wherein the tonicity of the solution is adjusted to a range of about 230 to about 490 mosmol/kg solvent.

16. The method of claim 15, wherein the tonicity of the solution is adjusted to a range of about 274 to about 309 mosmol/kg solvent.

17. The method of claim 12, wherein the tonicity of the solution is adjusted by adding an amino acid to the solution.

18. The method of claim 17, wherein the amino acid used is glycine.

19. The method of claim 1, wherein the trialkylphosphate is tri-n-butyl phosphate and the detergent is selected from polysorbate 80 and sodium cholate.

20. The method of claim 1, wherein the solution has a pH between about 3.5 and about 6.0 during step a).

21. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001 M, a pH between about 3.5 and about 5.0, an antibody concentration of about 5% wt./wt., and a maltose concentration of about 10% wt./wt.

22. The preparation of claim 21, wherein the pH is about 4.25.

23. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001, a pH between about 3.5 and about 5.0, an antibody concentration of about 10% wt./wt., and a glycine concentration of about 0.2 M.

24. The preparation of claim 23, wherein the pH is about 4.25.

ABSTRACT

Method of reducing the anticomplement activity (ACA) resulting from viral inactivation treatment of a solution of antibodies, the method comprising contacting the solution with a trialkylphosphate, such as tri-n-butyl phosphate, and a detergent, such as sodium cholate, under conditions sufficient to reduce substantially the virus activity, and then incubating the solution under controlled conditions of time, pH, temperature, and ionic strength such that the anticomplement activity is reduced to an acceptable level. In a preferred embodiment, the ACA is reduced to less than 60 CH_{50} units/mL, the incubation is for at least about ten days at a pH from 3.5 to 5.0, the temperature is maintained within a range of 2 to 50° C, and the ionic strength of the solution is less than about 0.001 M.

B

DECL[illegible]ATION FOR PATENT APPLICAT[illegible]

Docket Number (Optional)
MSB-7232

As a below named inventor, I he[illegible] declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN, the specification of which is attached hereto unless the following box is checked:

☐ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
NONE			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Number)	(Filing Date)	(Status – patented, pending, abandoned)
NONE		

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: James A. Giblin, Reg. No. 25,772

Address all telephone calls to James A. Giblin at telephone number _____

Address all correspondence to James A. Giblin
Bayer Corporation
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Berkeley, CA 94701

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name) WILLIAM R. ALONSO
Inventor's signature William R. Alonso Date 9-18-95
Residence Cary, North Carolina NC Citizenship U.S.A.
Post Office Address 1524 Dirkson Court
Cary, North Carolina 27511

Full name of second joint inventor, if any (given name, family name) _____
Second Inventor's signature _____ Date _____
Residence _____ Citizenship _____
Post Office Address _____

☐ Additional inventors are being named on separately numbered sheets attached hereto.

(2-92) Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

MSB-7232

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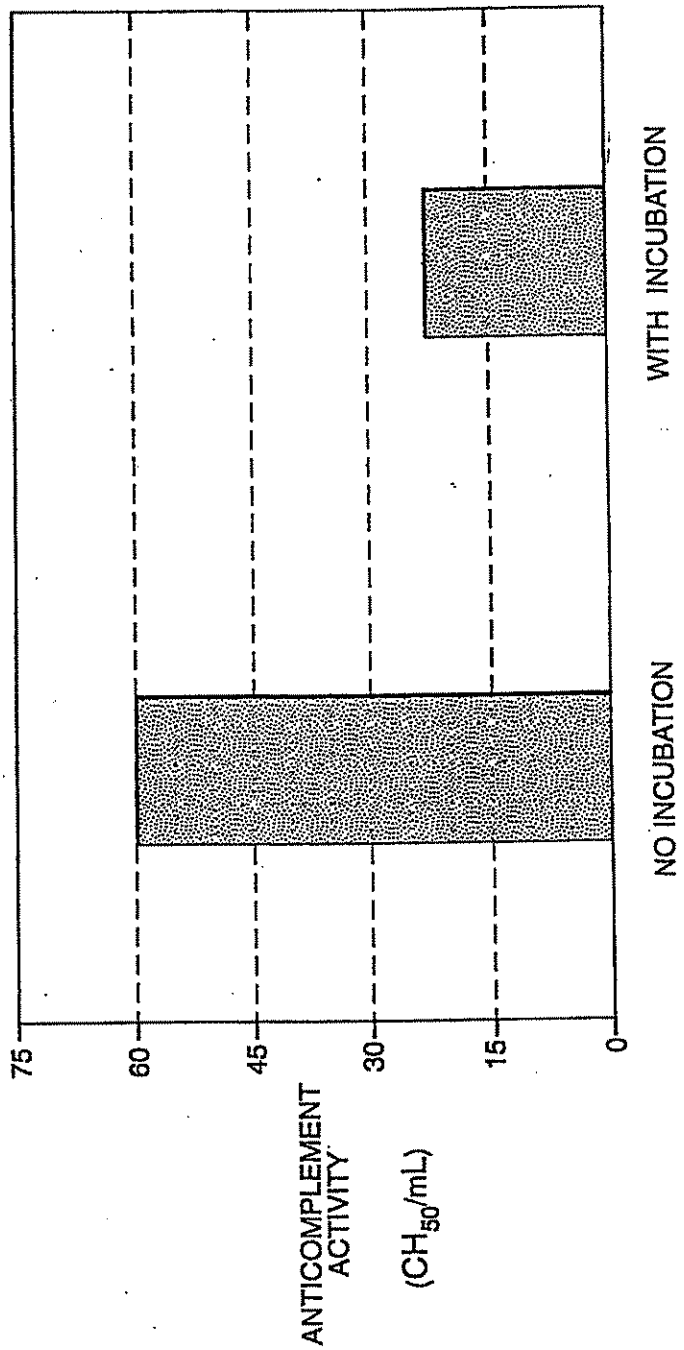
532211

APPROVED	D.C. FIG.
BY	

Replaced by drawing of 5/14/96



Office Action Summary	Application No. 08/532,211	Applicant(s) Alonso
	Examiner Yvonne Eyler	Group Art Unit 1806

☐ Responsive to communication(s) filed on _____.

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-24* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-24* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— *SEE OFFICE ACTION ON THE FOLLOWING PAGES* —

Patent and Trademark Office
O-326 (Rev. 9-95) Office Action Summary Part of Paper No. 2

Claims 1-24 are pending in the application.

1. Claims 1, 3-6, 10, 21, and 23 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is vague and indefinite in the recitations "a given level of anticomplement activity" and "an acceptable level suitable for intravenous administration." The metes and bounds of what is defined by a "given level" or an "acceptable level" cannot be determined.

Claim 10 is vague and indefinite because it recites an "ionic strength less than about 0.001" but fails to define what type of measure or what type of units are associated with the figure 0.001.

Claims 3-6, 21 and 23 refer to a solution comprises either 5% wt/wt antibody or 10% wt/wt antibody. This limitation is vague and indefinite because it is unclear to what the wt/wt refers. If the antibody is in an aqueous solution, then in would be wt/vol. If it is measured as wt/wt, it is unclear what the antibody is being measured with respect to.

2. The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

3. Claims 1-24 are rejected under 35 U.S.C. § 103 as being unpatentable over Tenold (U.S. # 4,396,608) in view of Neurath et

al (U.S.# 4,540,573), Mitra et al (U.S.#4,762,714), and Joy Yang et al (Vox Sang 67:337-344, 1994).

Tenold et al teach the modification of immune serum globulin (a solution of antibodies purified from blood plasma) to reduce anticomplement activity so that the solution may be safely administered intravenously. The starting material for Tenold's modifications is human immune serum globulin purified by Cohn's method, either fraction II or III. The starting solution is diluted in a physiologically acceptable carrier so as to obtain a protein or antibody concentration of about 0.5-20%. The pH of the solution is than adjusted, and maintained, at about 3.5 to 5.0 with a physiologically acceptable acid such as hydrochloric acid. The temperature range is maintained at about 0-20 degrees C. The ionic strength of the solution is adjusted to less than 0.001 and the tonicity is adjusted, without altering the ionic strength, by addition of an amino acid such as glycine or a carbohydrate such as maltose. The specific osmolality of the final isotonic solution is not specified but the acceptable range to maintain tonicity would be well known and conventional to one of ordinary skill in the art. The final product obtained by the method of Tenold is an immune serum globulin, maintained at a controlled pH, temperature, ionic strength and tonicity so as to generate a monomeric solution of antibodies with a reduced anticomplement activity rendering the solution safe for intravenous administration. While Tenold does not incubate the solution at

the given pH and temperature prior to the adjustment of ionic strength and tonicity, the pH and temperature are maintained throughout the procedure and the antibody solution is stored for up to six months under the defined controlled parameters. (see column 4 line 24 to column 8, line 54) The measured anticomplement activity of the immune serum globulin produced by Tenold's method is 3 mg protein per CH50 unit, which is less than 30,45 and 60 units. Tenold differs from the instant invention in that the starting material is not pre-treated to inactivate any infectious agents which may be present.

Neurath et al teach a method for the inactivation of infectious virus present in blood or blood derived solutions while maintaining the activity of proteins contained in the composition. The method comprises treating the solution with a trialkylphosphate, for example tri-n-butyl phosphate and a wetting agent such as a detergent for example polysorbate 80 or sodium deoxycholate followed by removal of the inactivating agents and optional further processing of the product. The starting material for the method of Neurath et al may include fraction II or III of the Cohn purification to obtain immune serum globulin which is virus free (see the abstract, column 1 lines 5-20, column 4 lines 50-60, column 6 lines 40-61, column 7 to 8 and column 9 lines 19-25)

Mitra et al discuss the need to produce virus-free immune serum globulin solutions to assure that active viruses are not

transmitted to patients. They follow the inactivation of spiked fraction II and III samples as the immune serum globulin solution is purified. They further specify that in order to obtain an immune serum globulin which is safe and effective for IV administration, the anticomplement activity of the solution must be reduced. They further specify that this may be accomplished through careful pH and ionic strength control (see column 1).

Joy Yang et al disclose a third-generation immune serum globulin for IV administration which includes a deliberate virus inactivation step of treatment with a solvent/detergent, specifically tri-n-butyl phosphate/polysorbate 80) (see the abstract and p.338, column 1). Joy Yang et al further discuss the desirability of retaining full Fc functions in a immune serum globulin preparation, including complement activity. Various assays are presented which evaluate the retention of Fc functions following the solvent/detergent treatment. An hemolysis test indicates that, in their system, the complement mediated lysis of erythrocytes is not affected by the solvent/detergent virus inactivation step, see p. 339, column 2). Joy Yang et al do not teach the further adjustment of the immune serum globulin product to reduce anticomplement activity, but do stress the importance of full complement activity to the effectiveness of an immune serum globulin solution.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method and

resultant immune serum globulin product of Tenold et al by pretreatment of the antibody solution with a solvent/detergent as taught by Neurath et al or Joy Yang et al to ensure inactivation of infectious virus which is taught to be desirable by Mitra et al and to maintain a low anticomplement activity which is taught to be desirable by Mitra et al and Joy Yang et al.

Note, the references cited were discussed at length in the specification and therefore a copy has not been included with this action.

4. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yvonne Eyler, Ph.D. whose telephone number is (703) 308-6564. Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Toni R. Scheiner

TONI R. SCHEINER
PRIMARY EXAMINER
GROUP 1800

Yvonne Eyler, Ph.D.
February 5, 1996

Notice of References Cited	Application/Control No. 08/532,211	Applicant(s)/Patent Under Reexamination ALONSO, WILLIAM R.	
	Examiner eyler yvonne	Art Unit 1646	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-4396608	08-1993	Tenold	
	B	US-4540573	09-1985	Neurath et al.	
	C	US-4762714	08-1988	Mitra et al.	
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Joy Yang, Y.H. et al. "Antibody Fc functional activity of intravenous immunoglobulin preparations treated with solvent-detergent for virus inactivation" Vox Sang, Vol. 67, pp. 337-344, May 17, 1994).
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

United States Patent [19]

Tenold

[11] **4,396,608**

[45] **Aug. 2, 1983**

[54] **INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN**

[75] Inventor: **Robert A. Tenold, Benicia, Calif.**

[73] Assignee: **Cutter Laboratories, Berkeley, Calif.**

[21] Appl. No.: **295,916**

[22] Filed: **Aug. 24, 1981**

[51] Int. Cl.³ C08L 89/00

[52] U.S. Cl. ..... 424/177; 424/85; 424/101; 260/112 B

[58] Field of Search ..... 424/85, 101, 86, 87, 424/177; 260/112 B

[56] **References Cited**

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4,093,606 6/1978 Coval ..... 260/112 B
4,186,192 1/1980 Lundblad 424/85

FOREIGN PATENT DOCUMENTS

47-37529 9/1972 Japan .

Primary Examiner—John C. Bleutge
Assistant Examiner—Patricia Short
Attorney, Agent, or Firm—Theodore J. Leitereg

[57] **ABSTRACT**

A composition is disclosed which comprises a solution in a pharmaceutically acceptable carrier of an immune serum globulin, said solution having an ionic strength and a pH to maintain the monomer content and the actual and latent anticomplement activity of the immune serum globulin such that the composition is intravenously injectable. Novel methods are disclosed for preparing the above composition.

14 Claims, No Drawings

INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates to pharmaceutical compositions comprising novel intravenously injectable immune serum globulin, to a process for its production and to its use to administer immune serum globulin intravenously for human therapy.

Intramuscularly injectable gamma globulin preparations are known. One such product is "HYPER-TET" (Cutter Laboratories, Inc., Berkeley, Calif.).

The usual intramuscular gamma globulin preparations cannot safely be administered intravenously because such administration causes an unacceptably high incidence of reactions, especially in agammaglobulinemic recipients. These reactions have been associated with a decrease in serum complement levels, apparently caused by complement binding by the administered gamma globulin. S. Barandun et al., *Vox Sang.* 7, 157–174 (1962). The ability of gamma globulin to bind complement, termed anticomplementary, is greatly increased as a result of denaturation brought about during the fractionation procedure, in particular by aggregation to high molecular weight species. The complement binding mechanism of these aggregates appears to be identical to that of antigen-antibody complexes. D. M. Marcus, *J. Immunol.* 84, 273–284 (1960). When the aggregates are removed by ultracentrifugation at 100,000 x gravity, a product low in anticomplement activity is obtained which is well tolerated upon intravenous injection. Barandun et al., supra.

Several approaches have been taken to the problem of rendering gamma globulin safe for intravenous administration. All of these are dependent on eliminating its anticomplement activity. Ultracentrifugation (cited above) is technically unfeasible, and the product so derived regains its anticomplement activity upon storage. Treatment of gamma globulin with the enzyme pepsin at pH 4.0 results in proteolytic cleavage of the molecule to give a fragment of about 10,000 molecular weight which has a sedimentation coefficient in the ultracentrifuge of about 5S,A. Nisonoff et al., Science, 132, 1770–1771 (1960). Even though this surviving fragment retains bivalent antibody activity and lacks anticomplement activity and is well tolerated and efficacious in intravenous administration, W. Baumgarten, *Vox Sang.* 13, 84 (1967), the therapeutic effect provided is of unacceptably short duration since it is rapidly excreted, having a circulating half-life of only 18 hours, perhaps somewhat longer in agammaglobulinemic patients, compared to 19.8 days for unmodified gamma globulin. E. Merler et al., *Vox Sang.* 13, 102 (1967); B. Jager, *Arch. Intern. Med.* 119, 60 (1967). Although the much reduced half-life of pepsin treated gamma globulin is probably due in part to the drastic reduction in size of the molecule, there are indications that the rate of catabolism of gamma globulin is related to specific properties of the portion of the molecule digested by pepsin. J. L. Fahey et al., *J. Exper. Med.*, 118, 1845–1868 (1963). This portion of the molecule remains intact in the present invention. An additional disadvantage of the pepsin treatment procedure is that the pepsin which remains present is of animal origin and can stimulate antibody production, particularly upon repeated administration. C. Blatrix et al., *Presse Med.* 77, 635–637

(1969). The use of plasmin of human origin avoids this difficulty and is the basis of a different process for preparation of intravenous gamma globulin.

Treatment of gamma globulin with human plasmin results in cleavage into three components of about 50,000 molecular weight. J. T. Sgouris, *Vox Sang.* 13, 71-(1967). When sufficiently low levels of plasmin are used, only about 15 percent of the molecules are cleaved, with 85 percent remaining as intact gamma globulin. Sgouris, supra. The intact gamma globulin remaining undigested shows little anticomplement activity and has been administered intravenously without adverse reactions. J. Hinman et al., *Vox Sang.* 13, 85 (1967). The material thus prepared appears to retain in vitro and in vivo protective activity. F. K. Fitzpatrick, *Vox Sang*, 13, 85-(1967). One disadvantage of this approach is that the plasmin cannot be completely removed. Thus, degradation continues even when the material is stored at 4° C.

Incubation of gamma globulin at pH 4.0 at 37° C. for various lengths of time has been observed to reduce the anticomplement activity to low levels. It has been suggested that this result may arise from a small quantity of serum enzyme present as an impurity in the gamma globulin. Blatrix et al., supra. As with the plasmin treated gamma globulin, this "pH 4.0 gamma globulin" has been found to regain anticomplement activity, upon storage, at an unpredictable rate, so that it is necessary to assay anticomplement activity before administration to a patient. J. Malgras et al., *Rev. Franc. Trans.*, 13, 173 (1970).

Both plasmin treated gamma globulin, Hinman et al., supra, and pH 4.0 gamma globulin, H. Koblet et al., *Vox Sang.* 13, 93 (1967); J. V. Wells et al., *Austr. Ann. Med.* 18, 271 (1969); Barandun et al., *Monogr. Allergy*, Vol. 9, 39–60 (1975), Barandun et al., *Vox Sang.*, Vol. 7, 157–174 (1962), have shorter half-lives in vivo than unmodified gamma globulin. For example, the half-life in normal patients of pH 4.0 gamma globulin is about 14 days, Koblet et al., supra, while the plasmin treated material shows a half-life of 16 days, Merler et al., supra.

The Centre National de Transfusion Sanguine (C.N.T.S.) in Paris has, by careful fractionation and filtration of gamma globulin from selected fresh plasma, produced an intravenously injectable gamma globulin with low anticomplement activity. Blatrix et al., supra; ibid., *Presse Med.*, 77, 159–161 (1969); M. Steinbuch et al., *Vox Sang.* 13, 103 (1967). It is apparently not totally devoid of anticomplement activity, as it must be administered carefully and reactions do occur in some patients. Cortisone may be given prior to injection to eliminate these reactions, but the apparent incomplete removal of anticomplement activity would seem to be detrimental to its widespread use.

The effects on anticomplement activity of reduction of disulfide linkages of gamma globulin followed by reaction with a blocking agent has been investigated in the prior art. S. Barandun et al., supra, found that treatment of a 7 percent solution of gamma globulin with 0.2 M cysteamine, followed by 0.2 M iodoacetamide, resulted in almost complete loss of anticomplement activity whereas treatment with cysteamine or iodoacetamide alone did not significantly decrease anticomplement activity. Because of the toxicity of iodoacetamide, these investigators did not pursue this approach to an intravenously injectable gamma globulin.

A modified immune serum globulin was described in U.S. Pat. No. 3,903,262. The immune serum globulin was rendered intravenously injectable by first reducing to —SH groups a portion of the disulfide linkages of the molecule and then alkylating the —SH groups. After the product was separated from the reaction mixture, it was sterilized. The so-produced material was intravenously injectable, substantially free from both actual and latent anticomplement activity, having substantially the biological half-life and spectrum of antibody activity of corresponding unmodified immune serum globulin.

Currently, there are several intravenously injectable gamma globulin products available outside the United States. One such product is INTRAGLOBIN of Biotest in Frankfurt. This product is made by beta-propriolactone treatment of gamma globulin (Stephan, *Vox. Sang.*, 1975, Vol. 28, pp. 422–437). The material has a molar concentration of sodium ion of about 0.18 and of chloride of about 0.27. The beta-propiolactone used in its preparation is suspected as a carcinogen.

Another intravenously injectable product is manufactured by Green Cross Corporation of Japan (U.S. Pat. No. 4,168,303). It is a lyophilized, natural gamma globulin preparation having an anticomplementary activity of less than or equal to 20 C'H50 units and 0.06–0.26 parts by weight of a neutral mineral salt such as sodium chloride.

The Swiss Red Cross has an immunoglobulin SCR for intravenous administration. SCR contains more than 80% of monomeric IgG and minor fractions of dimeric, polymeric, and fragmented IgG as well as traces of IgA and IgM. The distribution of IgG subclasses equals that of normal serum. The product is manufactured in lyophilized form and contains 3 g of protein, 5 g of saccharose and a small quantity of sodium chloride per unit. A diluent (100 ml) contains 0.9% sodium chloride.

VENOGLOBULIN (Green Cross Corporation of Japan) is prepared by treating gamma globulin with plasmin. It also contains 0.5 parts of a protein stabilizer (e.g. amino acetate) per 1 part by weight of plasmin treated gamma globulin. The product is distributed as a white powder and is dissolved in a diluent for use. The resulting solution is clear or slightly turbid and has a pH of 6.4–7.4.

An intravenously injectable gamma globulin has been developed by Schwab of Germany and contains 50 mg per ml immunoglobulin, 7 mg/ml glycin, and 7 mg/ml sodium chloride.

Schura of Germany manufactures an intravenously injectable gamma globulin by adsorption onto hydroxyethyl starch. The product is distributed as a solution having a pH of 6.7 and a conductivity of 450 mosm. and containing 2.5% glucose, 165 meq/l of sodium ion and 120 meq/l of chloride ion.

VEINOGLOBULINE is available from Institute Merieux of France. It is a plasmin-treated gamma globulin distributed as a lyophilized powder containing 5 g. of protein and enough glycine and sodium chloride to insure pH and stability. The diluent is 100 ml of water for injection containing 0.9 g. of sodium chloride or isotonic glucose.

U.S. Pat. No. 4,160,763, assigned to Behringwerke AG of Germany, is directed to an immunoglobulin for intravenous administration having reduced complement fixation made by treating an immuno globulin fraction with a low concentration of a sulfitolytic agent and/or phosphate which is sparingly soluble in water. The pH of the material is 7.0, and the product contains 0.85% sodium chloride and 2.5% (g/v) glycine prior to lyophilization.

Teijin Institute of Tokyo is the assignee of record of U.S. Pat. No. 4,059,571 for a novel immunoglobulin derivative. A water soluble composition for intravenous injection which contains the novel derivative is described. The derivative is the S-sulfonated product of cleaved interchain disulfide bonds of gamma globulin.

GLOVENIN, a pepsin-treated human immunoglobulin, is manufactured by Nihon Seigaku of Japan. Typically, a solution of the above product contains 50 mg/ml of pepsin-treated immunoglobulin, 2.25% (w/v) of aminoacetic acid, and 0.85% (w/v) sodium chloride.

Yamanouch Seiyaku is the distributor of GLOBULIN V, a dried pepsin-treated human immunoglobulin (500 mg) containing 225 mg of aminoacetic acid and 85 mg of sodium chloride. For intravenous administration the dried product is disolved in 10 ml of water for injection.

SUMMARY OF THE INVENTION

I have discovered an unmodified intravenously injectable immune serum globulin having an ionic strength and a pH such that the monomer content of the immune serum globulin is greater than about 90% and the actual and latent anticomplement activity is maintained such that the immune serum globulin is intravenously administrable to a broad spectrum of patients.

The product of my invention is prepared by a method wherein an immune serum globulin (ISG) is solubilized to yield a solution of a certain protein concentration. The pH of this solution is adjusted, and the ionic strength of the solution is reduced, to a level such that the monomer content of the ISG is greater than about 90% and the actual and latent anticomplement activity is such that the ISG product is rendered intravenously injectable. The pH and ionic strength are maintained at the above levels during protein concentration adjustment, sterilization, filling into final containers, and the like.

One advantage of the ISG of the invention is that it is intravenously injectable thus avoiding the problems associated with intramuscularly injected material. Furthermore, the present product is substantially free from chemical modification such as occurs in reduction-alkylation, beta-propiolactone treatment, and the like.

An important feature of the product of the invention is that it is substantially free of actual and latent anticomplement activity and also substantially free of polymeric material or "aggregates". Particularly, the product of the invention exhibits enhanced stability over prior art preparations. The material may be kept at room temperature for long periods in the absence of additives with retention of its monomer content and lack of actual and latent anticomplement activity.

Another advantage of the invention is that the intravenously injectable ISG is virtually unchanged in physical measurements and biological functions. Thus, the antibody titers in the present material are not significantly different from the starting material.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The starting material for the process of this invention is unmodified human immune serum globulin. In the specification and claims the term "immune serum globulin" is used to define the substance also referred to in

the literature variously as gamma globulin, IgG and immunoglobulin G. It consists predominantly and preferably of at least about 85 percent of the 7S species of gamma globulin, which has a molecular weight of about 160,000. Any remainder is preferably 9S species, with a molecular weight of about 300,000. Both standard immune and hyperimmune serum globulins, e.g., tetanus, rabies and hepatitis immune serum globulins, can be employed, the modified product being immune and hyperimmune ISG, respectively. Thus, a suitable starting material for the process of this invention is Cohn's Fraction II or Fraction III filtrate. See Cohn et al., *J. Am. Chem. Soc.* 68, 459 (1946); Oncley et al., ibid., 71, 541 (1949).

Fraction II, by ultracentrifugation studies, is predominantly (about 85 percent) the 7S (sedimentation constant of 7) species of gamma globulin with an average molecular weight of 160,000. The remaining protein is essentially 9S material with a M.W. of about 300,000. Wet Fraction II paste (approximately 30 percent solids) is commonly lyophilized to obtain dry ISG powder which is then dissolved and prepared for intramuscular injection as a 16.5 percent sterile solution. Either the wet Fraction II paste or the dry ISG powder is a suitable starting material for the process of this invention.

Gamma globulin obtained by any process which has essentially the same composition of protein components as found in the Cohn Fraction II or Fraction III filtrate can be used as starting material in the present process.

Both standard immune serum globulin and hyperimmune serum globulin can be employed as starting materials. As is well known, the latter is produced from plasma or serum obtained from selected donors who have much higher titers for a specific antibody than is normally found in the average population. These donors have either been recently immunized with a particular vaccine or else they have recently recovered from an infection or disease. These high titer sera or plasmas are pooled and subjected to the usual Cohn fractionation procedures up to the point of isolating Fraction II. The Bureau of Biologics (BoB) antibody standards for hyperimmune serum globulins presently are based on products to be given intramuscularly. These standards are based on the assumption a standard intramuscular dose of the reconstituted globulin (1–10 ml) will be administered. Because the amount of antibody required to achieve a desired immunological response is substantially less when administered intravenously, it will be apparent the I.V. dose will be substantially less than the I.M. dose which will produce the same serum antibody titer. Thus, the dose of intramuscular ISG and hyperimmune serum globulin must be higher than that required to achieve the same serum antibody titer when globulin of the same antibody activity is administered intravenously.

The starting wet paste or lyophilized powder is dissolved in a volume of water or other physiologically-acceptable carrier to provide a protein solution of a concentration of about 0.5–20% preferably about 5 percent. If Fraction III filtrate is employed, the aqueous solution must be concentrated by conventional techniques to the desired protein concentration. Any protein concentration may be used in this method; however, the above-recited range is preferred from a practical standpoint.

After the protein has been dissolved or concentrated, the solution is adjusted to a pH of about 3.5 to 5.0 preferably about 3.8 to 4.2, by addition of a physiologically-acceptable acid such as hydrochloric acid. In general, the pH is adjusted to a point whereat the monomeric material in the protein solution is maintained at a maximum. However, the pH must not be so low as to result in gelation. The temperature should not be harmful to the ISG material. Good results are obtained within the temperature range of about 0°–20° C. It is not necessary to hold the so-adjusted material for any period of time prior to the next step; however, the material may be held, if desired, without detrimental effects.

Following pH adjustment the protein solution is treated to reduce its ionic strength to a level at which the monomer content of the ISG preparation is greater than about 90%, preferably greater than about 95%, and more preferably greater than about 98%, and the actual and latent anticomplement activity is such that the ISG preparation is intravenously injectable. For this purpose the actual anticomplement activity should be greater than about 2 mg protein/C'H50 unit. The non-specific complement binding capacity of the product is determined using optionally titered complement and hemolysin. The complement binding capacity, known as anticomplement activity, is reported as mg protein product capable of inactivating (binding) one C'H50 unit. One C'H50 unit is defined as the amount of protein capable of inactivating 50% of complement in an optionally titered complement and hemolysin system.

The ionic strength ($\Gamma/2$) of the solution should be such that the product as a 5% protein solution has a nephelometric reading less than about 15 NTU (National Turbidity Units), preferably less than about 2 NTU. The ionic strength ($\Gamma/2$) is defined as follows:

$$\Gamma/2 = \frac{\Sigma\{[C^+]^2(Z^+)^2 + [C^-]^2(Z^-)^2\}}{2}$$

where

C^+ = cations including metal ions such as Na^+, K^+, Ca^{+2}, Mg^{+2}, and the like,

C^- = anions including halide ions such as Cl^-, Br^-, carboxylic acid salt ions such as acetate or citrate ions, and the like,

Z^+ = the charge of C^+, and

Z^- = the charge of C^-.

Preferably, the ionic strength, as defined, is less than about 0.001. The above treatment may be effected by standard procedures such as ultrafiltration, diafiltration, dialysis, etc., or combinations thereof. For example, the protein solution at the appropriate pH may be diafiltered with at least five volume exchanges of water, usually about 4–8 volume exchanges, to reduce the ionic strength to at least about 0.001. During this treatment the concentration of peptides and other impurities such as alcohol are also reduced, generally to trace amounts.

After or during the above treatment, the pH is measured and maintained within the range of about 3.5–5.0.

The protein concentration of the so-treated material is next adjusted to the level desired in the final product, such as, for example, 5%, 10%, 15%, and so forth. This adjustment is accomplished by conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Again, the pH of the preparation is maintained within the range of about 3.5–5.0, preferably about 3.8–4.2.

Next, the ISG preparation is treated to render it tonic, i.e., to render it compatible with physiological conditions or render it physiologically acceptable upon

injection. In this respect it is important to note that tonicity must be obtained without raising the ionic strength (as defined above) of the preparation. This end is achieved by adding to the ISG preparation an amount of an amino acid, such as glycine and the like, or a carbohydrate, such as maltose, dextrose, fructose, and the like, or a sugar alcohol such as mannitol, sorbitol, etc., or mixtures thereof sufficient to achieve tonicity. Thus, for example the ISG preparation may be mixed with about 10% maltose (on a weight to volume basis) to render the preparation tonic.

After the above adjustment the product is sterilized, usually by sterile filtration through appropriate media, and then filled into final containers. It is also possible to lyophilize the sterile ISG product after filling into final containers. For I.V. use the lyophilized material is dissolved in medically-acceptable water prior to injection. If the product has not been made tonic prior to lyophilization, the lyophilized material must be dissolved in a diluent containing medically-acceptable water and one of the aforementioned substances in an amount to render the preparation tonic.

The ISG of this invention is primarily intended for intravenous administration although the ISG preparation may also be administered intramuscularly if it contains the appropriate excipients. The composition aspect of this invention therefore relates to pharmaceutical compositions comprising a solution, in a pharmaceutically acceptable aqueous carrier adapted for intravenous administration, of an intravenously injectable ISG of this invention. The ISG is substantially pure. The ISG is present in these solutions in any concentration, either suitable for immediate I.V. administration or after dilution, e.g., with water or diluent as mentioned above, to acceptable levels, e.g., about 1–18 percent solution, preferably about 1–15 percent and more preferably about 10 percent for immediate administration, and about 16 percent for dilution prior to administration. The ISG can be administered intravenously alone or in combination with or in conjunction with other blood products, e.g., whole blood, plasma, Plasma Protein Fraction, fibrinogen, clotting factors such as Factor VIII, Factor IX concentrate, and so forth, and albumin.

In its method of use aspect, this invention relates to the intravenous administration, usually to humans, of a pharmaceutical composition as defined above. The composition is administered in a conventional manner, e.g., in an amount which provides adequate therapeutic amounts of antibody. For a 16.5 percent protein solution, about 1–25 ml is the customary single dose. Administration of subsequent dosages is usually within 1–3 weeks, depending upon the severity of the illness and the time of exposure thereto.

As mentioned above the products of the invention may be incorporated into pharmaceutical preparations, which may be used for therapeutic purposes. However, the term "pharmaceutical preparation" is intended in a broader sense herein to include preparations containing a composition in accordance with this invention used not only for therapeutic purposes, but also for diagnostic and reagent purposes as known in the art; for tissue culture wherein organisms such as viruses for the production of vaccines, interferon, and the like, are grown on plasma or on plasma fractions, e.g., Cohn Effluent II+III, Cohn Fraction IV, Cohn Fraction V, and so forth; etc. The pharmaceutical preparation intended for therapeutic use should contain a therapeutic amount of the present composition, i.e., that amount necessary for preventative or curative health measures. If the pharmaceutical preparation is to be employed as a diagnostic or a reagent, then it should contain diagnostic or reagent amounts of such composition. Similarly, when used in tissue culture or a culture medium the medium should contain an amount of such composition sufficient to obtain the desired growth.

The gamma globulin of this invention is substantially free from anticomplement activity, both immediate and latent.

Antibody titer is not significantly different from the starting unmodified gamma globulin, i.e., it is normal or hyperimmune, e.g., tetanus or rabies hyperimmune globulin, depending on the antibody titer of the starting ISG. The antibody molecules are bivalent, as indicated by their ability to precipitate with antigen.

Another characterizing feature of the ISG of this invention is its absence of proteolytic activity. It is known that some samples of ISG form fragments when stored. Such fragmentation is due to proteolytic digestion by a contaminating enzyme often presumed to be plasmin. Fragmentation is undesirable since it causes a decrease in the amount of active antibody in solution. The process of this invention sharply decreases the proteolytic activity in ISG to undetectable levels or at most to trace levels.

A primary and important characteristic of the present product is its stability. The product may be stored for extended periods of time without significant, if any, change in its antibody activity, monomer content, clarity, lack of anticomplement activity and so forth. For example, sterile, final container material prepared in accordance with this invention has been stored at room temperature on the shelf for greater than 6 months without significant changes in the above-mentioned qualities. This stability is obtained through pH and ionic strength adjustments as described above. The art heretofore has not recognized the relationship between pH and ionic strength on the one hand and intravenous injection on the other. As mentioned above, treatment of gamma globulin at pH 4 is known. However, the so-treated material was then returned to about pH 7 for administration to patients. Furthermore, addition of salts such as sodium chloride was employed to obtain tonicity.

A related benefit of the product of the present invention is its lack of buffer capacity. The present product is surprisingly administrable at pH 3.5–5.0. However, since the ionic strength has been reduced to a very low level, there is very little disruption, if any, of the physiological pH such as that which would occur with the administration of a material essentially buffered at pH 3.5–5.0 by the presence of salts.

EXAMPLES

The invention is demonstrated further by the following illustrative examples.

EXAMPLE I

The pH of Fraction III filtrate (2100 l.) from the Cohn fractionation scheme (Cohn et al, supra) was adjusted to 4.0 by addition of 1 N HCl. Approximately 40 l. of HCl was added at a rate of less than one liter per minute with thorough mixing. The Fraction III filtrate was then metered into an ultrafiltration system. Ultrafiltration and diafiltration were used to reduce the alcohol concentration as rapidly as possible while holding the product temperature less than 10° C. Cold distilled

water was used to maintain a constant volume of approximately 350 liters. Flux rates as high as 20 l. per minute were observed. When all the Fraction III filtrate had been concentrated to about 5% protein and the product alcohol concentration had been reduced to less than 8%, seven volume exchanges were performed using cold distilled water. The product temperature was permitted to drift as high as 20° C. The immune serum globulin solution was then concentrated to 8% protein and drained from the ultrafiltration system; 120 l. of 8% immune serum globulin was recovered in a clear "water-like" state. This material had an ionic strength of 0.001 (as determined by calculation) and a pH of 4.2. An aliquot of this material was made tonic with 10% maltose at 5% protein. This was filled into 250 ml bottles (60) for stability and other testing. Initial high pressure liquid chromatography (HPLC) results indicated a monomer level greater than 99%. This lot passed all typical testing for IGIV (Table 1). Several containers were stored at room temperature and after six months, HPLC results indicate the monomer content was still greater than 99%.

TABLE 1

HPLC Monomer (99.1%) Dimer (0.9%) Trimer (0) Void (0)	
Anticomplement Activity	3 mg protein per CH50 unit
PKA	11% of reference
Buffer Capacity	16.24 meq./L
Ultra-centrifuge	6.6S 90.8% 9.8S 9.2%
Nephelometer	1.5 NTU

A similar aliquot was made tonic by addition of glycine to a concentration of 0.2 M.

EXAMPLE 2

An aliquot (6 l.) of the 120 l. of 8% immune serum globulin prepared in Example 1 was treated with 1 N HCl to obtain a pH of 4.0 and lyophilized.

Water for injection was added to this material to obtain a 5% protein concentration. The reconstituted material exhibited the following characteristics:

TABLE 2

HPLC Monomer (98.5%) Dimer (1.5%) Trimer (0) Void (0)	
Anticomplement Activity	3 mg protein per CH50 unit

What is claimed is:

1. A stable, sterile, intravenously injectable pharmaceutical composition comprising an aqueous solution of a therapeutic amount of an immune serum globulin, said solution having an ionic strength such that the solution at 5% protein concentration has a nephelometric reading less than 15 NTU, a pH of about 3.5–5.0, and a physiologically-acceptable tonicity.

2. The composition of claim 1 which further includes other blood products.

3. The composition of claim 1 which includes a material selected from the group consisting of carbohydrates, sugar alcohols, and amino acids in an amount sufficient to render physiologically-acceptable tonicity to the solution.

4. The composition of claim 3 wherein the carbohydrate is maltose.

5. The composition of claim 3 wherein the amino acid is glycine.

6. A method for treating immune serum globulin which comprises

(a) forming an aqueous solution of an immune serum globulin,

(b) adjusting the pH of said solution to about 3.5–5.0 by addition of a physiologically acceptable acid,

(c) treating the solution to reduce its ionic strength ($\Gamma/2$) while maintaining the pH of said solution at 5% protein concentration has a nephelometric reading less than 15 NTU, and

(d) adjusting the tonicity of the solution to a physiologically acceptable level by addition of an agent selected from the group consisting of amino acids, carbohydrates, and sugar alcohols.

7. The method of claim 6 wherein the solution in Step a has a protein concentration of about 0.5–20% by weight.

8. The method of claim 6 wherein the pH is adjusted to about 3.8–4.2 in Step b and maintained thereat in Step c.

9. The method of claim 6 wherein the solution is diafiltered in Step c.

10. The method of claim 6 which further includes the step of

(e) sterilizing the solution.

11. A composition comprising the product of claim 6.

12. The method of claim 6 which further includes the step of lyophilizing the solution of Step c.

13. A composition comprising the product of claim 12.

14. A dry composition comprising immune serum globulin which upon solution in water has a pH of about 3.5–5.0 and an ionic strength ($\Gamma/2$) such that the solution at 5% protein concentration has a nephelometric reading less than about 15 NTU.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,396,608
DATED : August 2, 1983
INVENTOR(S) : Robert A. Tenold

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 10, claim 6, lines 21 - 24, correct step (c) to recite as follows:

--(c) treating the solution to reduce its ionic strength (Γ/2) while maintaining the pH of said solution at about 3.5 - 5.0, to a level such that the solution at 5% protein concentration has a nephelometric reading less than 15 NTU, and--.

Signed and Sealed this

Sixth Day of November 1984

[SEAL]

Attest:

GERALD J. MOSSINGHOFF

Attesting Officer

Commissioner of Patents and Trademarks

United States Patent [19]
Neurath et al.

[11] Patent Number: 4,540,573
[45] Date of Patent: Sep. 10, 1985

[54] UNDENATURED VIRUS-FREE BIOLOGICALLY ACTIVE PROTEIN DERIVATIVES

[75] Inventors: Alexander R. Neurath, New York; Bernard Horowitz, New Rochelle, both of N.Y.

[73] Assignee: New York Blood Center, Inc., New York, N.Y.

[21] Appl. No.: 514,375

[22] Filed: Jul. 14, 1983

[51] Int. Cl.³ A61K 39/00; A61K 35/14; A61K 37/00; C07G 7/00; C07C 103/52

[52] U.S. Cl. ..... 424/85; 260/112 B; 260/112 R; 260/121; 260/112.5 R; 424/101; 514/2; 514/6

[58] Field of Search ..... 260/112 B, 112 R, 121; 424/85, 101, 89, 177

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Primary Examiner—Thomas G. Wiseman
Assistant Examiner—Robin Lyn Teskin
Attorney, Agent, or Firm—Sprung Horn Kramer & Woods

[57] ABSTRACT

A mammalian blood protein-containing composition such as whole blood, plasma, serum, plasma concentrate, cryoprecipitate, cryosupernatant, plasma fractionation precipitate or plasma fractionation supernatant substantially free of hepatitis and other lipid coated viruses with the yield of protein activity to total protein being at least 80% is disclosed. The protein-containing composition is contacted with di- or trialkylphosphate, preferably a mixture of trialkylphosphate and detergent, usually followed by removal of the di- or trialkylphosphate.

34 Claims, 8 Drawing Figures

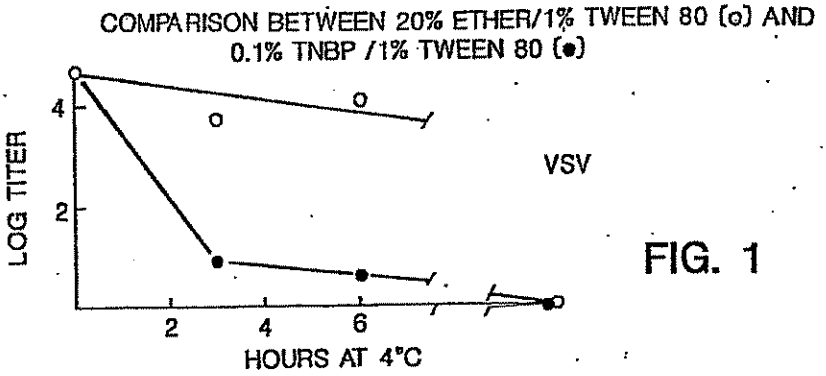

FIG. 1

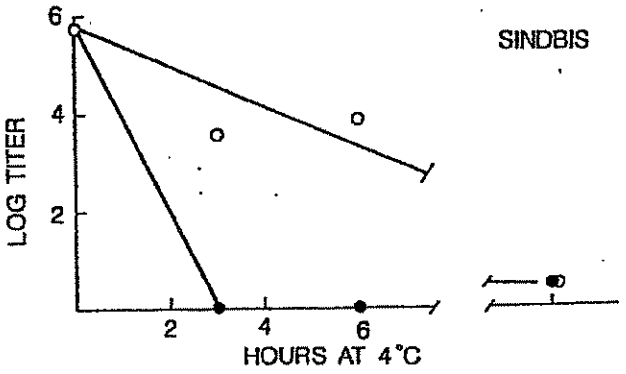

FIG. 2

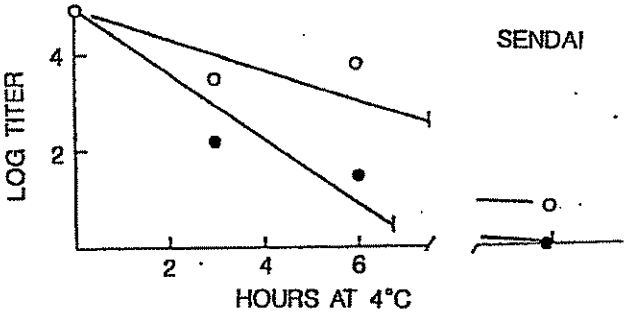

FIG. 3

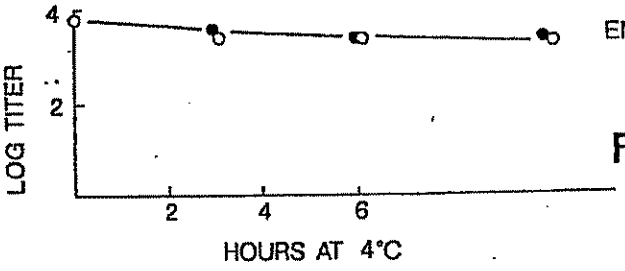

FIG. 4

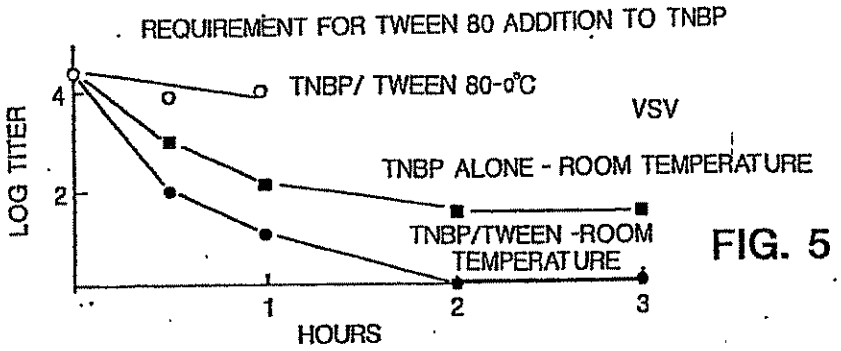

FIG. 5

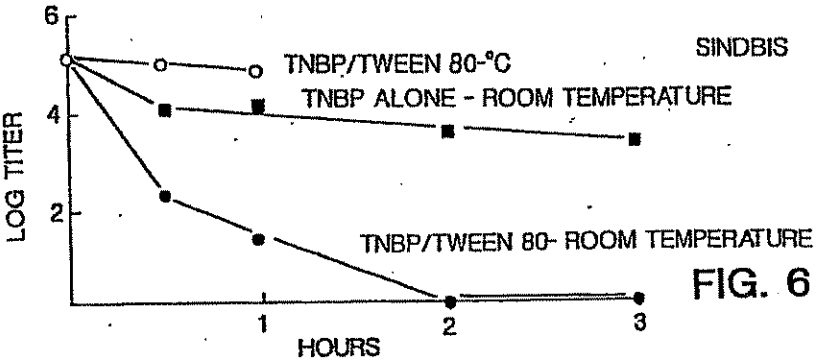

FIG. 6

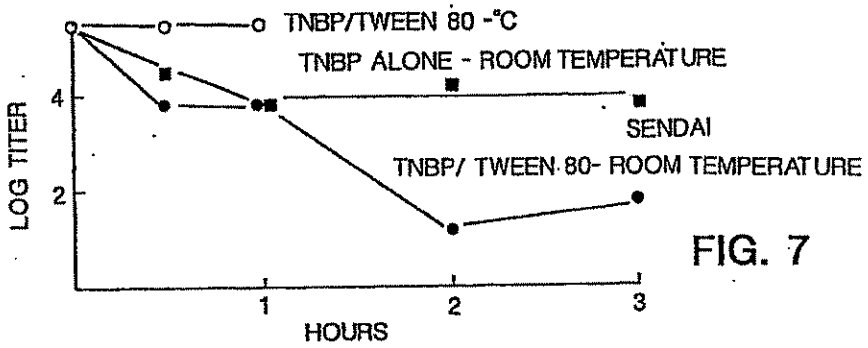

FIG. 7

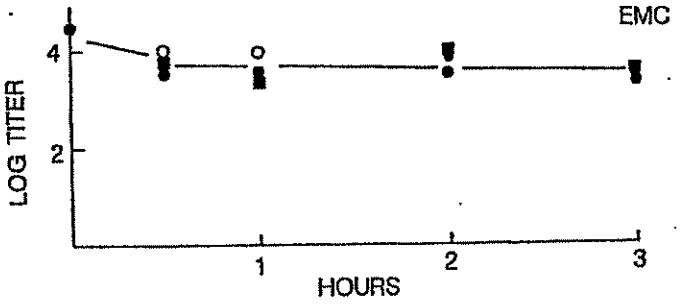

FIG. 8

o TNBP/TWEEN 80-°C
■ TNBP/TWEEN 80-ROOM TEMPERATURE
TNBP/TWEEN 80 ALONE-ROOM TEMPERATURE